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Lung metabolism, function, and morphology during hyperoxic and hyperbaric exposure

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ABSTRACT

Productivity resulting from the multi-disciplinary approach (cf. List of Personnel) we have been developing for studying O_2 toxicity is obvious. We have quite thoroughly defined (both qualitatively and quantitatively) the temporal sequalae of cardiopulmonary structural changes associated with onset, duration, and intensity of O_2 exposure as well as changes due to varying the rate of withdrawal from the hyperoxic environment. These studies in rats, rabbits, and hamsters have been extended to testing a variety of pharmacologic as well as dietary manipulations which alter tolerance to pulmonary O_2 toxicity and hypoxic pulmonary vasoconstriction. At present each bit of information obtained continues to direct us toward microsonal enzymes and serotonergie regulation. Our major goals for Year 05 involve studies directed at the involvement of these systems in the oxygen-related insults.

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OBJECTIVES

During the Year 04 specific efforts have been made to improve the cohesiveness of our program. We have extended our investigations to hypoxia as well as hyperoxia in order to provide a more clear understanding of pulmonary structure-function relationships throughout a wider continuum of inspired fractional O_2 concentrations. Objectives for Year 04 were as follows:

I. To develop an *in vitro* model suitable for quantitating pulmonary vascular surface area.

II. To provide more inclusive dose-response type quantitative as well as qualitative descriptions of pulmonary oxygen toxicity as those relate to duration and intensity of hyperoxic exposure.

III. To investigate the role of anti-oxidant enzyme systems, namely superoxide dismutase and glutathione peroxidase as they relate to hypoxic and hyperoxic-induced structural changes in the lung.

IV. To more clearly describe the role of oxygen in the pharmacology of pulmonary neuroendocrine cells.

Progress to date on each of these objectives will be described in Sections I-IV of this report (cf. SUMMARY OF ACCOMPLISHMENTS). However, in addition to these Year 04 objectives, we have completed preliminary studies directed at determining the role of inspired oxygen in controlling pulmonary disposition of vasoactive substances, particularly angiotensin, vasoactive intestinal peptide, substance P and bombesin. These latter studies incorporate technologic approaches developed in our lab throughout the past 5 years. It is our intention that such a systematic experimental design present the scientific community with a more rational approach complete with necessary

considerations for studying this non-respiratory function of the lung. These studies will be described in more detail in Sections V and VI (cf. SUMMARY OF ACCOMPLISHMENTS).

SUMMARY OF ACCOMPLISHMENTS

I. SURFACE AREA OF THE PULMONARY ENDOTHELIUM

A prerequisite to studies directed at quantitating pulmonary endothelial surface area as well as *a priori* for our investigations concerning the pulmonary disposition of vasoactive substances has been to develop a reliable approach for calculating residence or contact time in a single passage of blood through the pulmonary circulation. We repreatedly find that mean transit time and pulmonary blood volume participate in determining the extent to which a particular agent affects or is affected by the lung. The publication entitled "Non-parametric determination of the distribution of transit times in the presence of early recirculation from sampled indicator-diluation curves" describes progress to date in our laboratory on this problem. The paper is being submitted to Research in Basic Cardiology.

We are attempting to solve the problem of surface area exposure in a number of ways. Christiane Malcorps is carrying out this work in the Department of Pharmacology, University of Antwerp, Belgium. She has solved many problems related to: a) development of the isolated left-lower lung lobe of the dog as the appropriate model and b) non-radionuclide methods of determining endothelial cell kinetics of vasoactive substances (eg., epinephrine, norepinephrine, angiotensin). This latter part is the most difficult since endogenous fluxes must also be considered. She is coming to

Madison the 15th of April for 3 weeks. During this time we will work closely with the groups from Yale and Medical College of Wisconsin to plan a collaborative approach to solving the problem.

II. PULMONARY O_2 TOXICITY IN HAMSTERS.

Dr. Gonder has completed an extensive series of studies directed at describing the temporal dependency of lung damage as it relates to intensity and duration of oxygen exposure and duration of recovery from the exposure. These studies demonstrate that continuous 100% O_2 exposure elicits significant medial thickening of small pulmonary arteries, whereas exposure to 60% O_2 (3 weeks) is associated with a decrease in medial thickness. These studies have also provided information suggesting that optimal recovery from high O_2 exposure is obtained by gradual rather than abrupt return to normoxia following oxygen exposure. These data therefore demonstrate the importance of studying animals during the recovery from O_2 exposure. Several O_2 exposure regimes which appeared to be sub-lethal were associated with marked changes during the post-oxygen exposure period. These changes subsequently develop into symptoms similar to pulmonary O_2 toxicity characteristic of longer exposures.

With regard to the temporal aspects of O_2 toxicity and tolerance, we have completed an investigation in hamsters which demonstrates that pre-exposure to low levels of O_2 (60% O_2 for 3 wks) or pre-treatment with endotoxin (48 hrs prior to O_2 exposure) provide partial protection against lung damage normally produced by continuous exposure to 100% O_2. In an attempt to determine the mechanisms of protection by these pre-treatments, preliminary studies have been completed in which we have studied O_2- and endotoxin-induced alteration of pentobarbital sleeping time; an indirect determinant of microsomal enzyme

activation. These preliminary studies demonstrate that both oxygen and endotoxin elicit changes in pentobarbital sleeping time; the direction of change being species specific. Based on these studies we are continuing to define the role of microsomal enzyme activation as it relates to O_2 toxicity in a more sophisticated biochemical manner (cf. Year 05 Proposal).

III. EFFECTS OF VITAMIN E AND/OR SELENIUM DEFICIENCY ON DEVELOPMENT OF PULMONARY CHANGES ASSOCIATED WITH HYPEROXIA AND HYPOXIA

The rational for using dietary Vitamin E and selenium deficient states in hyperoxia relates to their role in anti-oxidant mechanisms. The purpose of the hypoxic intervention combined with these dietary manipulations was twofold. Firstly, we wished to provide quantitative enzyme profiles coupled with pulmonary morphometric changes over a wider O_2 continuum. Secondly, we proposed to determine whether or not hypoxic-induced alterations in specific anti-oxidant enzyme activities (superoxide dismutase--both copper-zinc and manganese forms and glutathione peroxidase) could underlie the 'apparent' protection against pulmonary O_2 toxicity afforded to animals pretreated with hypoxic environments. One major accomplishment arising from this study was to incorporate the biochemical technology required for studying the subject of mutual interest, i.e. oxygen toxicity. Pertinent literature citations and a thorough description of the methods used in this study have already been forwarded to you in the Year 05 Proposal. We have completed the analysis of circulating plasma glutathione peroxidase and preliminary lung tissue enzyme analysis. These data coupled with determinants used to describe cardiac hypertrophy and vascular responsivity to changes in inspired oxygen, i.e. right ventricululat/left ventricular plus septum weight and medial thickness of small pulmonary vessels respectively have presented very interesting findings. It appears that selenium deficiency potentiates hypoxic pulmonary

vasoconstriction and that dietary manipulations which elevate blood and tissue levels of reduced glutathione may prtect abainst pulmonary O_2 toxicity.

IV. PHARMACOLOGY OF PULMONARY NEUROENDOCRINE (NE) CELLS

Although several years were required to develop and validate the techniques used in studying NE cells; benefits of these efforts are evident from the progress of this past year. In attempting to ascribe a function for these 'paraneurons' we have raised more questions than we have answered. We have however clearly demonstrated that NE cells respond to both hypoxia and hyperoxia in a manner dependent upon both intensity and duration of exposure. These responses are assessed in terms of alterations in NE cell numbers as well as their serotonin content. Presumably, a change in pulmonary 5-HT levels could contribute to hemodynamic manifestations of O_2 toxicity as well as hypoxic pulmonary vasoconstriction. This working hypothesis led to our studies involving monocrotaline toxicity and pharmacologic attenuation of hypoxic pulmonary vasoconstriction (cf. APPENDICES).

Data obtained from these studies further implicates the microsomal enzyme system and serotonin in O_2-induced lung damage and pulmonary responsivity. In order to define the interelationships among 5-HT, microsomal enzymes and endotoxin as they relate to pulmonary oxygen toxicity, Ken Burhop has completed preliminary studies (6 expts) in awake sheep suggesting that pharmacologic antagonism of serotonergic mechanisms may alter the pathologic sequalae associated with acute endotoxemia. Coordination of these studies with those of Dr. Gonders' in Year 05 should generate data supporting this hypothesis.

V. THE ROLE OF INSPIRED OXYGEN ON PULMONARY DISPOSITION OF VASOACTIVE SUBSTANCES

Our primary objective was to determine whether or not hypoxia alters angiotensin converting enzyme (ACE) acivity *in vivo*. The first series of studies are complete. We have clearly demonstrated that ACE activity assessed in terms of a systemic pressor bioassay in awake sheep is independent of F_IO_2. The data also indicate a temporal relationship since acute hypoxia (less than 3 hrs) is associated with an apparent attenuation of the A-I pressor response which is only apparent when the dose-response curves for A-I and A-II are plotted on a drug weight basis rather than using molar representation.

VI. PULMONARY DISPOSITION OF VASOACTIVE PEPTIDES

Circulating levels of vasoactive peptides (eg. Bombesin, BN: vasoactive intestinal peptide, VIP; Substance P, SP) are elevated during endotoxemia produced by *E. coli* endotoxin (preliminary studies during Year 04 in this lab). Subsequent studies (presently in progress) were designed to investigate the effects of these peptides on hemodynamics and vascular permeability in awake sheep in an attempt to determine if vasoactive peptide activation underlies the cardiovascular responses of actue endotoxemia. The data suggest that a significant amount of circulating BN is extracted in passage through the pulmonary circulation. The extraction ratio appears to increase with increasing plasma levels of BN. Unlike in the dog, cat, rabbit or chicken, blood levels of BN greater than 1000 pM/L have negligible vasomotor activity in sheep. A bimodal response in lung lymph flow and the lymph to plasma protein ratio (L/P) suggest that BN may cause premeability changes. In contrast, VIP produces systemic hypotension and mild pulmonary hypertension

accompanied by a progressive increase in permeability. Further studies are being conduced to determine whether the permeability change associated with VIP infusion is direct or indirect.

PUBLICATIONS

MANUSCRIPTS IN PRESS (cf. APPENDIX A)

A Modification for Preparing the Chronic Lung Lymph Fistula in Sheep. M. J. Brown, D. F. Erichsen, R. Helgerson, and J. A. Will. (Journal of Applied Physiology)

Dynamics of the Neuroendocrine Cell-Regulatory Peptide System in the Lung. I. M. Keith and J. A. Will. (Exptl. Lung Research)

MANUSCRIPTS SUBMITTED (cf. APPENDIX B)

Non-parametric Determination of the Distribution of Transit Times in the Presence of Early Recirculation from Sampled Indicator-Dilution Curves. R. Rodriguez, C. Malcorps, J. A. Will, E. N. Lightfoot. (Research in Basic Cardiology)

Endotoxin-induced alterations in Pulmonary Endothelial Permeability Norepinephrine Removal, and Hemodynamics in Awake Sheep. D. F. Erichsen, C. Malcorps, M. Brown, R. A. Proctor, J. R. Starling and J. A. Will (Journal of Applied Physiology)

Pharmacologic Attenuation of Hypoxia-Induced Arterial Hypertrophy in Rat Lungs. I. M. Keith. J. A. Will and K. E. Weir. (Journal of Applied Physiology)

Monocrotaline Intoxication in the Rat: Prevention of Cardiac Changes by Dipyridamole and Sulfinpyazone. I. M. Keith, J. A. Will, E. K. Weir and R. Huxtable.

MANUSCRIPTS IN PREPARATION (cf. APPENDIX C)

Effect of Hyperoxia on Hemodynamics, Permeability and Amine Removal in Awake Sheep. D. F. Erichsen, J. A. Will and R. H. Demling.

Acute Pulmonary Artery Hypertension Produced by Distension of the Main Pulmonary Artery Compared with Acute Hypoxia in Awake Sheep. D. F. Erichsen, C. Juratsch, M. Brown and J. A. Will.

Adverse Reaction to the Injection of Premixed Autogenous Blood and Indocyanine Green in Awake Sheep. D. F. Erichsen, C. Malcorps, and J. A. Will.

PRESENTATIONS

Breathing High-Temperature, Humidified Air Results in Changes Pulmonary Epithelial Cells. (Presented at FASEB, Atlanta, Spring, 1981)

The Acute and Chronic Effects of Hemorrhagic Shock on Pulmonary Capillary Endothelium. (Presented at FASEB, Atlanta, Spring, 1981)

Temporary Inhibition of the Pulmonary Vascular Pressor Responses to Hypoxia and Prostaglandin F_{2a} by Diamide. (Presented at American College of Cardiology, Spring, 1981).

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ANIMAL USE STATEMENT

All animal studies and preparations used in the experiments outlined in this report have been designed within the guidelines for the CARE AND USE OF LABORATORY ANIMALS. Permission and supervision of such studies has been approved by RARC, the appropriate commission at this University.